

Behavioral and Metabolic Responses to Emersion and Subsequent Reimmersion in the Freshwater Bivalve, *Corbicula fluminea*

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Abstract. When exposed to air, the freshwater bivalve, *Corbicula fluminea*, displayed valve movement behaviors, such as mantle edge exposure, wider gaping “ventilatory” response, and an escape or “burrowing” response. The proportion of the emersion period spent in these behaviors, relative to valve closure, increased with decreasing temperature. Emersion at 35°C inhibited valve movement behaviors, whereas emersion in a nitrogen atmosphere stimulated ventilatory activity. High rates of aerial oxygen uptake (\dot{M}_{O_2}) were associated with initial valve opening and ventilatory behaviors, and lower \dot{M}_{O_2} occurred during bouts of mantle edge exposure. Heart rate was affected by temperature, but not by mantle edge exposure. Heart rate increased during burrowing and ventilatory behaviors suggesting a hydraulic function for hemolymph. Emerged *C. fluminea* had short bursts of heat production followed by longer periods of lower heat flux when measured by direct calorimetry. The mean heat production rate was 1.11 mW (g dry tissue)⁻¹, significantly higher than the mean value for clams exposed in a nitrogen atmosphere, 0.50 mW (g dry tissue)⁻¹. On reimmersion, *C. fluminea* showed no significant “oxygen debt” until after three days aerial exposure. The bursts of activity, while emersed, may be the

result of periodic renewal of oxygen stores followed by immediate oxygen use.

Introduction

Aerial exposure of marine intertidal bivalves results in a variety of behavioral and metabolic responses (McMahon, 1988; Shick *et al.*, 1988). In general, bivalves inhabiting the shore will either close their valves while emersed and undergo anaerobic metabolism (especially lower shore species such as *Mytilus edulis*, *Cerastoderma glaucum*, Boyden, 1972a; Widdows *et al.*, 1979), or their valves will periodically gape allowing the maintenance of an aerobic metabolism (predominantly higher shore species, *Cerastoderma edule*, *Geukensia demissa*, Boyden, 1972a; Widdows *et al.*, 1979).

The intertidal environment is characterized by emersion periods that are predictable and of short duration. In contrast, bivalves inhabiting the shallow regions of freshwater lotic and lentic environments are subject to periods of emersion that are highly unpredictable in their duration, timing, and temperature. Freshwater bivalves can withstand periods of aerial exposure ranging from a few days to months, and will consume oxygen while in air (Dietz, 1974; McMahon and Williams, 1984). To survive such prolonged emergence, bivalves must balance two opposing requirements: to maintain contact with the atmosphere for gas exchange, while minimizing evaporative loss of water.

The Asian freshwater clam, *Corbicula fluminea* (Müller), is commonly found in shallow lakes and streams throughout the United States (McMahon,

Received 3 November 1989; accepted 20 February 1990.

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Abbreviations: \dot{M}_{O_2} —oxygen consumption rate; f_h —heart beat frequency; TW—tapwater; \dot{q} —weight-specific heat flux.

1982). A recent invader of freshwater, *C. fluminea* has higher tolerances to emersion than its estuarine relatives, but a relatively low tolerance among other freshwater bivalves (McMahon, 1979; Byrne *et al.*, 1988). It displays several behavior patterns when aerially emersed, such as mantle edge exposure and valve gaping, which are associated with aerial oxygen uptake (McMahon, 1979, 1983; McMahon and Williams, 1984).

In this study, we examined the effects of temperature and hypoxia on the behavioral responses of *C. fluminea* to emersion. Exposure of emersed clams to a N₂ atmosphere allowed us to discriminate between responses to aerial exposure and hypoxia. We examined metabolic responses, including heart rate, aerial oxygen uptake, and heat flux on emersed clams. In addition, the responses to reimmersion after varying periods of aerial exposure were observed.

Materials and Methods

Animals

Specimens of *C. fluminea* were collected, either from the Clear Fork of the Trinity River at its outflow from Lake Benbrook, Tarrant Co., Texas, or from the littoral region of the south shore of Toledo Bend Reservoir on the Texas-Louisiana border. Specimens were maintained, unfed, in aquaria containing either aged tapwater (TW) or artificial pondwater (Dietz and Branton, 1975) at 22–24°C for at least one week prior to use. The animals ranged in size from 19 to 43 mm shell length; 3.8 to 21 g total wet weight; and 0.15 to 0.79 g dry tissue mass. Dry tissue mass was about 8% of total wet body mass, but varies with season (Williams and McMahon, 1989).

Behavioral measurements

The effects of temperature on valve movements of aerially exposed specimens of *C. fluminea* were determined on 5 clams at each of 3 temperatures, 15, 25, and 35°C. In addition, the effects of exposure to a severely hypoxic (N₂) atmosphere (P_{O₂} ≈ 1 torr) were examined. Measurement of valve movements was made by gluing a monofilament line to a point 1–2 mm from the leading edge of a valve. The opposite valve was attached to a syracuse dish by gently embedding the bivalve, on its side, in modeling clay. The syracuse dish with its attached bivalve was placed inside a 45-ml glass jacketed chamber sealed by a rubber stopper. About 1 ml of distilled water was added to the chamber to maintain relative humidity near saturation. The line was threaded through an 18-gauge hypodermic needle passing through the rubber stopper and was attached tautly to the lever of a displacement transducer. The amplified output was directed to a strip chart recorder. The lever was counterweighted so

that little force was exerted on the line attached to the valve, and the line was coated with silicone grease to provide a gas seal but to also allow movement. Temperature inside the chamber was maintained at 15, 25, or 35 ± 0.1°C by means of a circulating water bath connected to the glass jacket of the respiration chamber. A period of temperature equilibration (30–60 min) was allowed before recording. Depending on the treatment temperature, experiments continued for 24–150 h. The major categories of valve movement behavior were identified by simultaneous observation of behaviors and the tracings.

A hypoxic atmosphere was achieved by flushing the chamber with N₂ gas at a high rate (300 ml/min) for the first 10 min, and at 50–75 ml/min during the experiment. The gas was appropriately temperature equilibrated and humidified before being introduced into the chamber. The P_{O₂} in these chambers was not routinely measured but we have recorded about 1 torr after the 10-min flushing period.

Heart rate

The heart rate of aerially exposed clams was measured simultaneously with valve movements under the three temperature treatments. Measurement of heart beat rate (f_h) was accomplished by a modification of the method of Dietz and Tomkins (1980), a non-invasive technique that records the shadow of the beating heart by means of a photocell attached to the outside of the shell. The photocell (silicon selenium; 5 × 5 mm) was positioned over the heart and allixed by a small piece of modeling clay or glue to the valve. The clam was prepared for valve movement recording, as described above, and the leads from the photocell were threaded through a hole bored in the stopper and then sealed with rubber cement. The photocell current output was amplified with a Keithly microammeter (100 nA full scale), and was input to an amplifier/chart recorder. Light from a fiber optic lamp was directed through the clam from outside the chamber and adjusted above ambient illumination until the recorder deflection, caused by the movement of the heart, was maximized. Only when the clam was performing burrowing behaviors, were the tracings difficult to interpret.

Aerial oxygen consumption

Aerial oxygen consumption rates (at 25°C) of clams exposed under normoxic conditions, and of clams after an exposure to hypoxic environments, were recorded. The aerial oxygen consumption rate (\dot{M}_{O_2}) was measured by the method of McMahon and Williams (1984). Moreover, valve movements and f_h were recorded simultaneously. Clams were prepared for valve movement and

f_h recording as outlined above. Specimens were scrubbed to remove organisms adhering to the shell that might interfere with \dot{M}_{O_2} determinations. A polarographic oxygen sensor (Yellow Springs) was inserted through the rubber stopper sealing the chamber so that its tip was positioned approximately halfway into the chamber. Other openings drilled through the rubber stopper for leads were sealed. After a 30-min equilibration period, simultaneous recordings were made of valve movement, f_h , and P_{O_2} . Reliable determination of \dot{M}_{O_2} could only be made over a period of <24 h as the sensor fluid would need to be replaced. Although \dot{M}_{O_2} measurements were attempted on fifteen specimens, only recordings of individuals displaying valve movements were used in the analyses. \dot{M}_{O_2} was calculated from the decline in percent oxygen saturation and expressed as $\mu\text{mol (g dry tissue}\cdot\text{h)}^{-1}$. Sensor drift was measured in an empty chamber and corrections applied to the \dot{M}_{O_2} calculations.

Direct calorimetry

Metabolic heat production by clams exposed in air or nitrogen gas was determined. The heat flux (mW) by emersed clams was measured by direct calorimetry, over periods of 24 to 168 h, with the LKB ThermoMetric 2277 Thermal Activity Monitor microcalorimeter (Suurkuusk and Wadsö, 1982; Gnaiger, 1983; Gnaiger *et al.*, 1989). Small clams (shell length 1.9–2.3 cm) were glued to a specially molded plastic platform (10 × 10 × 10 mm). This platform was designed to present the clam lying on its side in the metabolic chamber (the orientation used previously), while suspending the clam above a 1-ml reservoir of distilled water placed in the chamber to maintain relative humidity near saturation. A 25-cm³ metabolic chamber was used in all experiments. Rates of heat dissipation were recorded by subtracting the output of a blank chamber (4 ml distilled water, the approximate thermal equivalent mass of the contents of the experimental chamber) from that of the experimental chamber, and recording the result on a strip-chart recorder. As the time-response curve of this larger chamber was not instantaneous, but approximates a first order exponential function (Gnaiger, 1983), the rates of heat dissipation derived from the experiments were averaged over 10-min periods and corrected to give instantaneous heat flux readings.

Heat production of the control chamber (4 ml distilled water replacing the clam) was determined after every two experiments, and an instrument calibration was performed every four experiments, or at least once a week. To determine the effects of emersion in a nitrogen atmosphere, the chamber was flushed for 30 min with humidified N₂ carried in capillary tubing incorporated into the cap of the metabolic chamber containing the experimental animal.

Aquatic \dot{M}_{O_2} on reimmersion

One hundred specimens of *C. fluminea*, individually marked and weighed (± 0.0001 g), were aerially exposed in desiccators above a layer of water maintaining relative humidity at near saturation, at 25°C. Five individuals, picked at random, were removed after 1, 2, 3, 5, and 6 days of emersion and used to determine the aquatic \dot{M}_{O_2} upon reimmersion. These clams were immersed in dechlorinated, aged tapwater, and allowed to open their valves and commence siphoning activity for 5 min. The immediate \dot{M}_{O_2} was determined by placing the clams individually into a sealed, temperature controlled respiration chamber (65 ml volume; $25 \pm 0.1^\circ\text{C}$) filled with aerated TW. The clam was supported on a nylon mesh platform above a magnetic stirbar. The decline in chamber dissolved oxygen was measured with a pre-equilibrated oxygen sensor (Yellow Springs) connected to a strip-chart recorder. Rates of oxygen consumption were determined on the basis of the first 10% decline in air saturation, which was usually accomplished in 5–10 min after the method of McMahon and Russell-Hunter (1977). The clams then were returned to aerated TW, and the aquatic \dot{M}_{O_2} remeasured after a total reimmersion period of 1 h to detect any temporal changes in respiratory responses of reimmersed individuals.

Data analysis

Data are expressed as mean \pm SEM, and n = the number of animals. Differences were considered significant at $P < 0.05$ with Student's *t*-test, or a one-way ANOVA followed by Duncan's Multiple Range tests.

Results

Behavioral responses to emersion

On emersion, specimens of *C. fluminea* displayed four categories of behavior (Fig. 1). The first was the closed condition with valves clamped shut and no tissues exposed to the environment. The second condition was mantle edge exposure with the valves parted slightly (1–2 mm) and portions of the leading edge of the mantle exposed along the complete extent of the gape. The mantle edges were moist or fused with a hardened mucus over the surface, and no opening into the mantle cavity was evident. On many occasions, the mantle was extended over the edge of the valves, exposing more mantle tissue.

The other two behavioral categories of valve movement on emersion were less common (Fig. 1). After a period of mantle edge exposure, the valves and mantle would part further, forming an opening into the mantle cavity. This position would be maintained for a few minutes followed by rapid valve closure; the opening and closing of valves could continue for some minutes giving

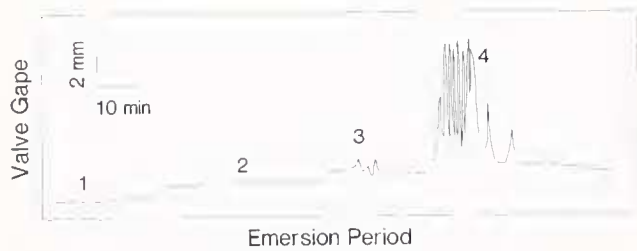


Figure 1. An amalgamation of several recordings to demonstrate the four categories of valve movement behavior in emersed *Corbicula fluminea*. 1. Valves closed. 2. Valves gaping slightly with little valve adduction, characteristic of mantle edge exposure. 3. Valves gaping wider with small medium frequency adductions, often associated with "ventilatory" movements. 4. Valves widely gaping with high frequency adductions indicative of the "escape" or "burrowing" behavior.

the appearance of a form of "ventilation" (category 3 in Fig. 1). The fourth behavior (rare) was a parting of the valves, and an extension of the foot. While the foot was extended, the valves would shut on the foot, then open, and the foot would extend further. This behavior would be repeated until the foot was extended maximally and touching the substratum. This activity resembles the "burrowing" behavior of immersed specimens of *C. fluminea*, and is interpreted as an escape behavior.

Although the patterns of behavior varied extensively between clams, the general progression of valve movement behaviors was similar for all. It began with a period of valve closure which lasted from 8.08 to 17.42 h (11.94 ± 1.98 ; $n = 4$) at 15°C, from 7.20 to 27.55 h at 25°C (15.44 ± 3.57 ; $n = 5$), and [when valve movement behavior was noted (only 3 of 10 cases)] from 0.33 to 6.52 h at 35°C (3.94 ± 1.86 ; $n = 3$). After the initial period of valve closure, bouts of mantle edge exposure were occasionally interspersed with short periods of ventilatory and, rarely, burrowing behaviors. The duration of this period was variable, but ranged from 36.16 to 203.59 h (126.31 ± 35.66 ; $n = 4$) at 15°C, from 64.78 to 93.38 h at 25°C (82.71 ± 6.19 ; $n = 5$), and 9.00 and 27.55 h at 35°C ($n = 2$). Following the period of valve movement, the valves remained closed to the end of the experiment, or death.

We determined the percentage of time spent by each clam in each of the behavioral categories with the times spent in ventilatory and burrowing behaviors combined. These values were averaged for each temperature treatment (Table I). An analyses of variance on the transformed values (arcsine of square root of the percentage as a proportion) showed that temperature had a significant effect on the relative time spent in the behavioral categories. The most striking was the inhibition of valve movements at 35°C: only 20% of clams exposed at 35°C displayed any valve movement behavior. Clams exposed at 15°C spent significantly ($P < 0.05$) less time closed and

more time in the mantle edge exposure behavior than those clams emersed at 25°C.

Clams exposed to hypoxia in a N₂ atmosphere (25°C) after they began valve movements, spent $40.3 \pm 10.1\%$ ($n = 3$) of the time with mantle edges exposed, and $57.0 \pm 7.6\%$ ($n = 3$) in either ventilatory or burrowing behaviors. After a period of severe hypoxia, and while ventilatory movements were still occurring, the chamber was flushed with humidified air. The result was a significant change in the pattern of valve movement behavior; *i.e.*, clams exposed their mantles $84.6 \pm 1.0\%$ ($n = 3$) of the time and spent $12.1 \pm 4.2\%$ ($n = 3$) of the time in ventilatory behaviors.

Heart rate while emersed

The heart rate of *C. fluminea* was highly variable, and this was true also of emersed specimens of *C. fluminea*; *i.e.*, for an individual clam in one experiment, the highest values could be more than twice the lowest. Temperature had a significant effect on mean f_h (Table II) with an approximate Q_{10} of 2. As we were interested in the effects of valve movement behaviors on f_h , we determined the heart rate 5 min before the valve movement began, the rate at the onset of valve movements, and f_h at 5 min after valve movement began. Because of individual variability in f_h , the change in f_h was expressed as the fractional change relative to the f_h during the first 5 min of the valve movement (Table III). At 15 and 25°C, the occurrence of mantle edge exposure behavior (Fig. 1, category 2) resulted in no significant change in f_h . The onset of the ventilatory behaviors (Fig. 1, categories 3 and 4) resulted in no change in f_h ; however, there was a significant 35% drop in f_h 5 min after the onset of the ventilatory behavior at 15°C. At 25°C, 5 min after the onset of ventilation, f_h declined 13% (Table III). As valve move-

Table I

The effects of temperature on valve movement behaviors in emersed Corbicula fluminea

Temperature (°C)	Valves closed	Mantle edge exposed	Ventilatory and burrowing behaviors
15	29.5 ± 5.9 A	65.8 ± 5.5 A	4.7 ± 1.7 A
25	51.2 ± 4.2 B	43.5 ± 3.8 B	5.3 ± 0.9 A
35	90.5 ± 8.0 C	9.1 ± 7.7 C	0.4 ± 0.3 B

Dissimilar letters after values indicate significant differences between temperature treatments (one-way ANOVA; Duncan's Multiple Range Test; $P < 0.05$; arcsine of square root transformation). Values are the mean ± SEM percentage of the emersion period spent in each behavioral category; ventilatory and burrowing activity were combined ($n = 5$ for each temperature).

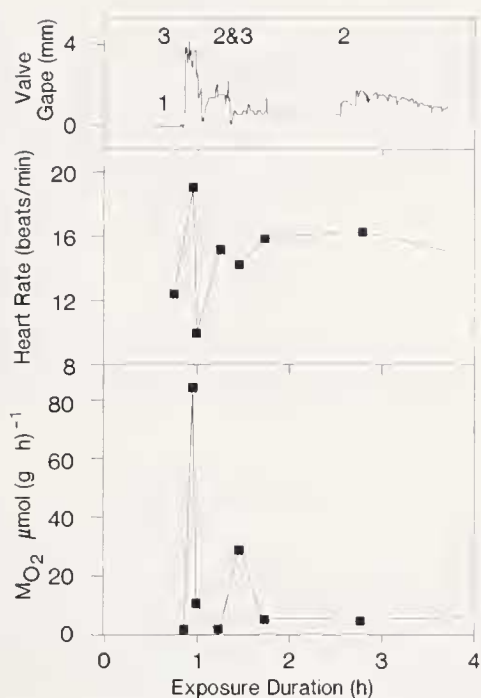


Figure 2. An example of valve movements (upper panel) with the same activity pattern notations as in Figure 1, simultaneous recordings of heart beat rate (middle panel) and aerial oxygen uptake (\dot{M}_{O_2} ; lower panel) in emersed *Corbicula fluminea*. The initial high rate of oxygen consumption is associated with valve opening and may be the result of oxygen depleted mantle cavity air mixing with the air in the respiration chamber. Heart rate increases during major valve movements.

ment behavior was rare at 35°C, no effects on f_h were measured.

Aerial \dot{M}_{O_2}

Of 15 attempts to record simultaneously \dot{M}_{O_2} , valve movements, and f_h in aerially exposed clams, measurable \dot{M}_{O_2} was recorded for only three animals. Figure 2 shows an example of O_2 uptake with the concurrent valve movement and f_h recordings. The pattern here, and in the other recordings, was an initial high rate of O_2 uptake coincident with the initial 2–3 min of valve movement, followed by a reduction in oxygen uptake during the period of mantle edge exposure. Further ventilatory movements were associated with elevated rates of oxygen depletion. Oxygen consumption during mantle edge exposure, although low, was measurably greater than when the valves were closed. Oxygen consumption rates integrated over periods of valve movement were 11.1 to 77.5 $\mu\text{mol } O_2 \text{ (g dry tissue} \cdot \text{h)}^{-1}$ (40.4 ± 19.6 ; $n = 3$), with most of the oxygen uptake occurring in short bouts. When the valves were shut, \dot{M}_{O_2} ranged from 0 to 0.5 $\mu\text{mol } O_2 \text{ (g dry tissue} \cdot \text{h)}^{-1}$. When ventilatory movements were initiated, the heart rate increased briefly then declined, even though valve movements continued.

Direct calorimetry

The pattern of weight-specific heat flux (\dot{q} ; $\text{mW} \cdot \text{g}^{-1}$) in emersed specimens of *C. fluminea* consisted of periods of steady heat flux interspersed with short bursts of relatively high \dot{q} (Fig. 3A; Table IV). The peak rates of heat dissipation were between 1.7 and 9 times the average “basal” aerial rate (heat flux between peaks); but the duration of these peaks was less than one hour, and clams had an average of one peak every 7.50 ± 0.89 h ($n = 12$) of emersion. The time of emersion (in seconds) times the average \dot{q} calculated from the continuous recordings over the entire period of emersion [$\text{mW (g dry tissue)}^{-1} = \text{mJ (g dry tissue} \cdot \text{s)}^{-1}$] yields the total energy expenditure over the period of aerial exposure.

Clams exposed to hypoxia did not display the bursts of peak activity noted in clams exposed in normoxia (Fig. 3B). The mean \dot{q} of nitrogen-emersed clams was not significantly different from the “basal” level of normoxic emersed clams (Table IV), but was significantly lower than the mean \dot{q} (= average energy expenditure) of normoxic emersed clams.

Aquatic \dot{M}_{O_2} on reimmersion

There was an increase in aquatic \dot{M}_{O_2} related to exposure time (ANOVA $F = 12.66$; $P < 0.001$). However, the effects of aerial exposure were not immediate (Fig. 4). Initial rates of oxygen consumption were not significantly different from control values even after three days of aerial exposure (Duncan’s Multiple Range). This suggests that the mussels were not accumulating a significant oxygen debt during three days of emersion. Initial oxygen consumption rose significantly ($P < 0.05$) from a control value of $61.7 \pm 5.7 \mu\text{mol (g dry tissue} \cdot \text{h)}^{-1}$ to $135.0 \pm 18.2 \mu\text{mol (g dry tissue} \cdot \text{h)}^{-1}$ after 5 days emersion (Fig. 4). \dot{M}_{O_2} continued to rise to $186.1 \pm 8.9 \mu\text{mol (g dry tissue} \cdot \text{h)}^{-1}$ after 6 days of exposure. There were no significant differences between initial and 1-h \dot{M}_{O_2} values at any time.

Table II

Effect of temperature on frequency of heart beat (f_h ; $\text{beats} \cdot \text{min}^{-1}$), in emersed *Corbicula fluminea*

	Temperature (°C)		
	15	25	35
Heart rate	8.4 ± 0.8 (3) A	14.7 ± 1.8 (6) B	35.8 ± 6.4 (3) C
Q_{10}	1.75	2.44	

Dissimilar letters after the values indicate significant differences (one-way ANOVA; Duncan’s Multiple Range; $P < 0.05$). Values for individual clams were averaged, and the grand mean \pm SEM (n) for each temperature is presented.

Table III

Change in heart beat frequency (f_h ; beats \cdot min $^{-1}$) associated with valve movements in emersed *Corbicula fluminea*

Temperature	Fractional change in f_h					
	n	Mantle edge exposed		n	Ventilatory and burrowing behavior	
		5 min before	5 min after		5 min before	5 min after
15°C	5	0.11 \pm 0.05	0.10 \pm 0.12	10	0.19 \pm 0.06	-0.35 \pm 0.07*
25°C	14	-0.02 \pm 0.02	0.01 \pm 0.03	9	0.14 \pm 0.03	-0.13 \pm 0.02*

The asterisks designate values within a temperature or behavior category significantly different from one another ($P < 0.05$). Values are fractional changes in f_h (\pm SEM) from 5 min before the onset of the behavior, compared to the f_h at the beginning of the behavior; and the fractional change of the f_h 5 min after the behavior had commenced, compared to the f_h at the onset of the behavior. A positive value indicates an increase in f_h .

By using the oxycaloric equivalent of -450 kJ/mol O_2 (Gnaiger *et al.*, 1989), heat production can be estimated from the \dot{M}_{O_2} of aquatic clams, and is approximately 27

J (g dry tissue \cdot h) $^{-1}$ [= 7.5 mW (g dry tissue) $^{-1}$]. Converting energy flux expressed in units of J \cdot s $^{-1}$ (= mW) to units of J \cdot h $^{-1}$, the absolute peak values of aerial heat flux were around 22 J (g dry tissue \cdot h) $^{-1}$, or 80% of the aquatic rate. The average peak heat dissipation rate was 9.4 J (g dry tissue \cdot h) $^{-1}$ (34% of the aquatic rate), the overall mean heat flux was 4.0 J (g dry tissue \cdot h) $^{-1}$ (15%) and the basal rate was 2.3 J (g dry tissue \cdot h) $^{-1}$ (9%).

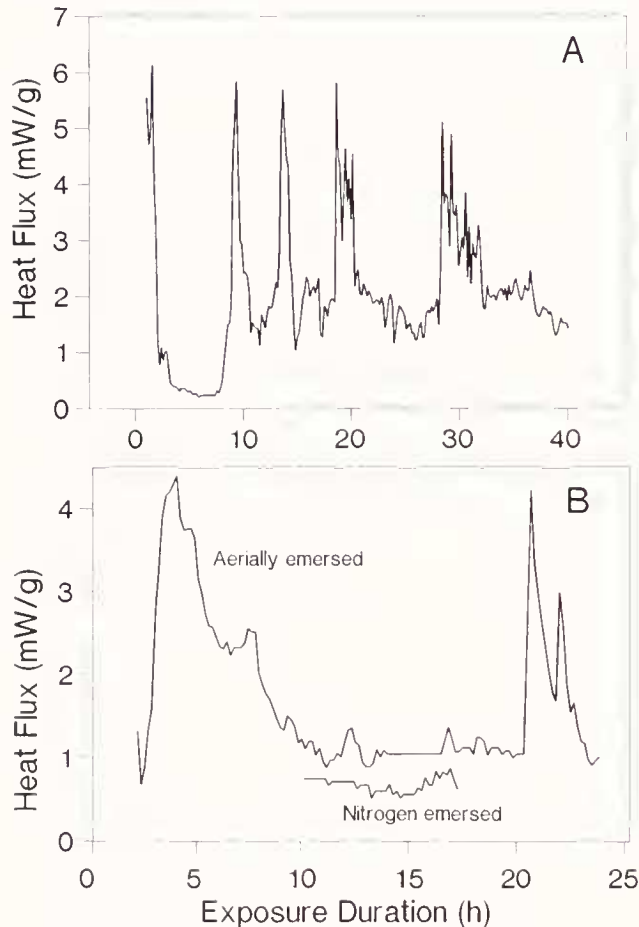


Figure 3. A. Example of the pattern of energy flux for an emersed *Corbicula fluminea* as measured by direct calorimetry. Note the bursts of higher rates of heat dissipation between periods of much lower activity. B. Part of a record of rates of heat dissipation in an emersed specimen of *C. fluminea* exposed in a normoxic and anoxic atmosphere. No burst activity was noted in nitrogen exposed clams.

Discussion

Corbicula fluminea displayed a suite of behavioral responses to emersion: mantle edge exposure; valve ventilatory behavior and burrowing response. These behavioral responses occupied a larger proportion of the total emersion period than had been estimated previously. McMahon and Williams (1984) reported a value for the proportion of time exposing mantle edge at 11.5% in *C. fluminea* emersed at 20°C. In the present study, valve

Table IV

Mean values of rates of heat dissipation (mW \cdot g $^{-1}$) in emersed *Corbicula fluminea*

	mW \cdot g $^{-1}$
<i>Normoxic emersed</i>	
Mean peak rate	2.55 \pm 0.29 (12)*
Mean basal rate	0.65 \pm 0.12 (12)
Mean heat flux	1.11 \pm 0.13 (12)*
<i>Nitrogen emersed</i>	
Mean heat flux	0.50 \pm 0.10 (3)

The asterisks indicate significant differences between mean rates of heat flux of nitrogen emersed clams and the aerially emersed bivalves ($P < 0.05$). Peak values are maximum rates sustained during bursts of activity. Mean basal values are average rates of heat dissipation measured during periods of no burst activity. Mean heat flux is the average of all values and approximates the mean energy expenditure of emersed clams. Values are expressed as the mean \pm SEM and the number of animals is given in parentheses.

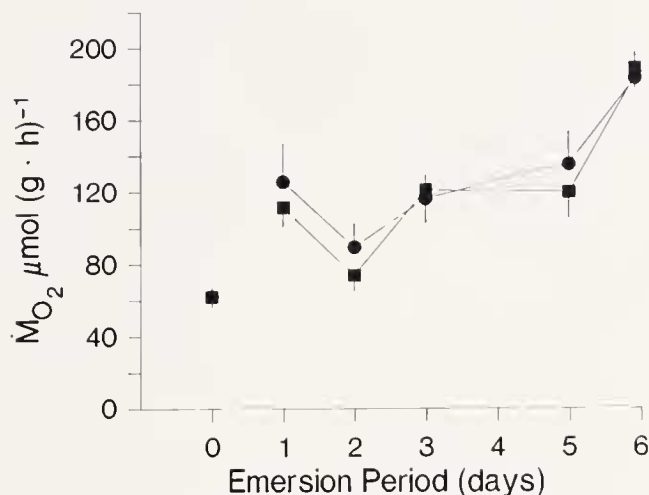


Figure 4. Rates of oxygen consumption (\dot{M}_{O_2}) of *Corbicula fluminea* on reimmersion after periods of aerial exposure. Circles represent initial values, within 5–15 min of the initiation of siphoning activity upon reimmersion. Squares are values measured one hour after siphoning activity commenced. The unconnected points represent corresponding initial and 1 h control \dot{M}_{O_2} measurements in tapwater acclimated clams. Bars are standard errors of the means and $n = 5$ animals for each point.

movements in emersed clams occupied 70% and 49% of the exposure period at 15 and 25°C, respectively. A similar pattern of valve movement behaviors while emersed has been reported for the high estuarine mangrove corbiculid, *Polymesoda erosa*. In *P. erosa*, ventilation of the mantle cavity air space occurred at irregular intervals, interspersed with periods of mantle edge exposure and valve closure (McMahon, 1988).

These behaviors in *C. fluminea* seem to be associated with aerial oxygen uptake (this study; McMahon and Williams, 1984). However, the highest rates of aerial oxygen consumption took place during the first few minutes of valve opening in what was referred to as ventilatory behavior, with lower rates during mantle edge exposure. This is consistent with the hypothesis of a periodically renewed, mantle cavity oxygen store (McMahon and Williams, 1984; Pamatmat, 1984). When the valves first open, the oxygen depleted mantle cavity gas mixes with the air, causing a sudden decline in atmospheric P_{O_2} in the respiration chamber.

Aerial oxygen uptake has been reported for several freshwater bivalves. The unionid clam, *Ligumia subrostrata* was reported to have aerial oxygen uptake rates of between 21–23% of the aquatic rate (Dietz, 1974), and the sphaeriid, *Sphaerium occidentale*, have similar rates of aerial oxygen uptake (Collins, 1967). Heming and co-workers (1988) reported that the freshwater mussel, *Margaritifera margaritifera*, periodically gaped in air with the result that dissolved oxygen levels in the mantle

fluid were maintained at approximately half those of immersed bivalves.

Among the intertidal and estuarine bivalves, Lent (1968) suggested that air-gaping in *Geukensia demissa* was an adaptation for air breathing. Non-gaping intertidal bivalves vary from having no measurable aerial O_2 uptake to oxygen consumption rates of 4–17% of the aquatic rate (Widdows *et al.*, 1979). In contrast, the gaping bivalve species have an aerial \dot{M}_{O_2} similar to that in water, or have aerial rates of between 28% and 79% of the aquatic rate (Boyden, 1972a; Booth and Mangum, 1978; Widdows *et al.*, 1979). In general, higher shore bivalve species have higher rates of aerial O_2 consumption associated with valve gaping, whereas lower shore clams, emersed for short periods, tend to remain closed and consequently have lower aerial \dot{M}_{O_2} (McMahon, 1988).

Emersion of *C. fluminea* in a nitrogen atmosphere seemed to stimulate a wider gape response, indicative of ventilatory activity, and the higher level of activity was diminished on return to a normoxic environment. This observation suggests that clam ventilatory behaviors have a respiratory function and may maintain some level of aerobic metabolism while emersed. In addition, a ventilatory loss of CO_2 is also clearly associated with mantle edge exposure (Byrne, 1988) as well as with O_2 uptake, as demonstrated here. The ability to dissipate metabolic CO_2 and maintenance of acid-base balance may be as important as O_2 uptake in the ability of *C. fluminea* to tolerate emersion.

Emersed *C. fluminea* also displayed bursts of heat production interspersed among periods of lower “basal” or quiescent activity. Freshwater bivalves have endogenous rhythms of activity and changes in oxygen consumption (McCorkle *et al.*, 1979). Our calorimetry chamber did not allow concurrent measurements of valve movement. However, emersed specimens of marine bivalves have valve gaping patterns associated with aerial oxygen uptake and elevated heat dissipation rates that are qualitatively similar (Pamatmat, 1984; Widdows and Shick, 1985). The bursts of heat production we observed could have been associated with valve closure or foot movements. However, the duration of valve movement is usually of short duration, but the peak \dot{q} activities we measured was of greater duration making this possibility unlikely. A likely explanation is periodic ventilation and gas exchange during valve gaping episodes resulting in a short-term elevation of aerobic metabolism. Recharging spent phosphagen and ATP stores during short periods of aerobic metabolism would result in increased heat dissipation rates. After valve closure the O_2 availability would be decreasing, even if relatively quiescent, and clams would gradually become anaerobic. Before incurring a significant oxygen debt, however, another bout of valve opening and ventilation would occur. Perhaps the

gradual depression of metabolic rate is due to a decrease in body fluid pH (deZwaan 1983).

Peak values of aerial heat dissipation in *C. fluminea* approached 80% of the aquatic rate. The mean peak rate of heat dissipation was 34% and the overall mean was 15% of aquatic rates in this study. These data correspond well to the aerial O_2 consumption rate being about 20% of the aquatic rate in other freshwater bivalves (Collins, 1967; Dietz, 1974) and *C. fluminea* (McMahon and Williams, 1984). In a N_2 atmosphere, emersed *C. fluminea* did not display bursts of heat production but maintained a low level of \dot{q} similar to the quiescent periods during normoxic exposure. This suggests that the observed bursts of heat flux in normoxia are the result of aerial respiration.

In most cases where bivalves gape while exposed, there is a continuation of heart beat during emersion. Changes in heart rate during emersion are variable, from essentially no change of f_h during emersion in some species, to a distinct bradycardia on emersion in other species (Boydén, 1972b; Coleman and Trueman, 1971). There appears to be no consistent direct effect of valve movement on heart beat frequency; some bivalves show occasional changes in f_h associated with valve movements and others display a suppression of heart activity (Trueman and Lowe, 1971; Coleman, 1976; Dietz and Tomkins, 1980).

Although valve gaping is associated with a respiratory function in emersed bivalves, the importance of circulating blood in the delivery of oxygen to tissues is not certain. Mussel blood typically has no oxygen carrier, and Booth and Mangum (1978) found that only 14% of O_2 in the blood of *Modiolus demissus* was delivered to tissues, leading them to conclude that the circulatory system was not important in this regard. We noted that increases in heart rate in emersed *C. fluminea* were associated mainly with ventilatory and burrowing behaviors. Foot and muscular movements are facilitated by hydraulic pressure and increases in heart rate may be associated with a redistribution of hemolymph among blood sinuses. Alternatively, when clams periodically have bursts in metabolic activity while emersed, the increased f_h may be in response to the momentary increases in perfusion requirements.

When returned to water after three days of emergence, *C. fluminea* had aquatic oxygen consumption rates that were elevated when compared to pre-emersion rates. The increased \dot{M}_{O_2} was evident within 5–10 min of reimmersion and remained elevated 1 h later. Repayment of an "oxygen debt," characterized by an elevated \dot{M}_{O_2} , is commonly encountered in marine bivalves reimmersed after periods of aerial exposure (McMahon, 1988; Shick et al., 1988). Frequently, the size of the oxygen debt is proportional to the duration of the exposure period and is repaid during the first hour of reimmersion (Bayne et

al., 1976; deVooy and deZwaan, 1978; Widdows et al., 1979; Widdows and Shick, 1985). In contrast, oxygen consumption rate in resubmerged *C. fluminea* is not a direct function of exposure time and, indeed, \dot{M}_{O_2} rates did not rise significantly above pre-emersion values until after three days emersion. \dot{M}_{O_2} also did not decline significantly after one hour of reimmersion. Both observations suggest that the elevated \dot{M}_{O_2} observed after three days of emersion was not a typical oxygen debt repayment in *C. fluminea*. Rather, over moderate periods of emersion, *C. fluminea* appears to maintain a sufficient level of aerobic metabolism to be able to avoid dependence on anaerobic metabolism. This corresponds directly with the maintenance of ventilatory and valve movements during the early stages of emergence. Elevated oxygen consumption after three days of emergence may be associated with long-term catabolic and anabolic demands resulting from the emersion stress.

Corbicula fluminea has evolved an additional suite of respiratory and behavioral adaptations, compared to *P. erosa*, an estuarine member of the family Corbiculidae (McMahon, 1988). Although valve gaping and limited emergence tolerance time are similar to high intertidal clams, the novel responses of *C. fluminea* include the exposure of mantle edges alternating with short bouts of valve gaping and ventilatory behavior. These responses would allow rapid exchange of mantle cavity gasses, yet minimize evaporative water loss. After three days of emergence, *C. fluminea* shows evidence of increased O_2 demand on reimmersion, and mortality increases. Although *C. fluminea* does not have the emersion tolerance of the more ancient families of freshwater bivalves (unioids, sphaeriids), it is a successful inhabitant of lakes and streams. Its short-term physiological mechanisms, and high reproductive capacity allow this species to be successful in the variable freshwater habitats.

Acknowledgments

The research was supported by a Sigma Xi Grant-in-aid of Research to R.A.B., the University of Texas at Arlington Research Grants to R.F.M. and NSF grant DCB 87-01504 to T.H.D. E.G. was partially supported by the LSU Department of Zoology and Physiology Visiting Scientist Program and Fonds zur Förderung der wissenschaftlichen Forschung in Österreich, project J0011. This study was part of a dissertation submitted by R.A.B. to the Graduate School of Louisiana State University and A&M College in partial fulfillment of the Ph.D. degree.

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